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Anti-diabetes effect of chronic intermittent hypobaric hypoxia through improving liver insulin resistance in diabetic rats

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ABSTRACT

Aim: Cumulating evidence demonstrated that chronic intermittent hypobaric hypoxia (CIHH) had beneficial effects on the body. The present study was to investigate the anti-diabetes effect of CIHH in type-2 diabetic rats for the first time.
Main methods: Sprague–Dawley rats were randomly divided into 4 groups: control group (CON), diabetes mellitus group (DM, induced by high-fat diet combined with low-dose streptozotocin), CIHH treatment group (CIHH, simulated 5000-m altitude, 6 h per day for 28 days), and diabetes mellitus plus CIHH treatment group (DM + CIHH). Histopathology of liver, systolic arterial blood pressure (SAP), blood biochemicals, glucose and insulin tolerance were determined. The expression of proteins associated with insulin signaling pathway as well as hypoxia induced factors were assayed.
Key findings: Diabetic rats showed impaired glucose tolerance, dyslipidemia, hepatic steatosis and hepatic insulin resistance in addition to increased SAP. However, SAP, serum triglyceride and cholesterol were decreased, and hepatic steatosis and insulin resistance were improved in DM + CIHH rats. Furthermore, the protein expression of glucokinase (GCK), insulin receptor substrates (IRS-1 and IRS-2), and HIF1α were increased, while the expression of phosphoenolpyruvate carboxykinase (PEPCK), was markedly reduced in DM + CIHH rats.
Significance: We conclude that CIHH treatment has anti-diabetes effects through ameliorating insulin resistance via hepatic HIF-insulin signaling pathway in type-2 diabetic rats.

1. Introduction

Diabetes mellitus, a common metabolic disease with rapidly increasing in prevalence in modern society, has become one of the most challenging public health problems in the world [1]. Type 2 diabetes mellitus (T2DM) is the major diabetes mellitus in human beings (>90% of diabetes mellitus) and a risk factor for cardiovascular diseases [2]. In addition, T2DM results in a lot of chronic complications including retinopathy, nephropathy, peripheral neuropathy and autonomic neuropathy [3]. Numerous researches showed that insulin resistance, defined as a reduced sensitivity of target tissues to insulin, played a vital role in the pathogenesis of T2DM [4]. In hepatic insulin resistance, the decreased hepatic glycogen synthesis and increased hepatic glucose production result in hyperglycemia and compensatory hyperinsulinemia [5], as well as dyslipidemia and hepatic steatosis, which further aggravates insulin resistance [6].

It has been found since 70's that populations living in high altitude have lower blood glucose levels or even lower incidence of type 2 diabetes mellitus than plain inhabitant [7,8]. High altitude hypoxia adaptation has been found to improve glucose tolerance in subjects with metabolic syndrome [9,10] and T2DM patients [11]. Chronic intermittent hypobaric hypoxia (CIHH) has been proved to have beneficial effects on the body such as cardio-protection and anti-hypertension [12]. Our previous study displayed that CIHH protected cardiovascular system against ischemia/reperfusion injury through multiple mechanisms or pathways [13–18]. It was also proved that CIHH regulated immune function and protected rat against collagen-induced arthritis [19]. Recently, we found that CIHH improved the abnormal metabolism, such as decreasing of fasting blood glucose and insulin resistance [20], as well as ameliorating nonalcoholic fatty liver disease (NAFLD) in fructose-induced metabolic syndrome rats [21].

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So we hypothesized that CIHH treatment had anti-diabetes effects through improving insulin resistance. The aim of present study was to investigate the anti-diabetes effects of CIHH and the mechanism in T2DM rats.

2. Materials and methods

2.1. Animals and CIHH treatment

Adult male Sprague–Dawley rats (body weight 250–300 g, provided by the Animal Center of Hebei Medical University) were randomly divided into four groups: diabetes mellitus group (DM), chronic intermittent hypobaric hypoxia treatment group (CIHH), diabetes mellitus plus CIHH treatment group (DM + CIHH) and control group (CON). DM and DM + CIHH rats were treated by high-fat diet for 4 weeks and intraperitoneal (i.p.) injection of streptozotocin (STZ) to induce T2DM. CIHH rats were exposed to hypobaric hypoxia simulating 5000-m altitude (P_B = 404 mm Hg, Po_2 = 84 mm Hg) for 4 weeks, 6 h/day in a hypobaric chamber. DM + CIHH rats were treated with 4-week CIHH after T2DM formation. All animals were housed in a temperature-controlled room (22 ± 1 °C) with a 12 h/12 h light/dark cycle and had free access to water and food. Systolic arterial blood pressure (SAP) were measured in conscious rats by a tail-cuff pressure meter (LE5001, Panlab) weekly, and body weight, amounts of food consumption, water intake and urinary volume of rats in 24 h were collected each week.

All the experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

2.2. Induction of T2DM model

The diabetic rat model was developed according to a modified method previously described [22]. Briefly, Type 2 diabetes was induced by feeding rats with a high-fat diet (20% lard, 20% sucrose, 10% yolk powder, 2.0% cholate, and 48% chow; 20.12 J/g) for 4 weeks followed by a single i.p. injection of low dose STZ (30 mg/kg, dissolved in citrate buffer, pH 4.2). Successful development of hyperglycemia was checked 1 week after STZ injection (5 weeks after the onset of high-fat diet feeding). The CON and CIHH rats were fed normal chow diet (13.77 J/g) and injected with vehicle. Fasting blood glucose (FBG) was assessed in STZ-injected rats using a One-Touch Ultra blood glucose meter (ACCU-CHEK, Germany). When FBG in rats ≥11.1 mmol/L, the diabetes model was thought successful and used in experiment.

2.3. Determination of blood biochemicals

Blood samples were collected from the angular vein of rats after 12 h fasting at the end of the ninth week of experiment (4 weeks after the onset of CIHH) and centrifuged at 3500 rpm for 10 min to get serum for assay. Fasting blood glucose, serum triglyceride (TG) and cholesterol (CHO) were measured by enzyme coupled colorimetric method with commercial kits (BioSino, China).

2.4. Glucose and insulin tolerance test

At the end of the ninth week of experiment (4 weeks after onset of CIHH treatment), animals were fasted overnight and then intraperitoneal injected with glucose (50% solution; 2 g/kg body weight), blood samples were taken at 0- (before injection), 30-, 60-, and 120-min after glucose injection. Plasma glucose was determined as described above. The area under the blood glucose curve (AUC) of glucose tolerance tests (GTTs) were calculated according to equation:

$$\begin{aligned}\text{AUC} = &(\text{blood glucose at } 0-\text{ min} + \text{blood glucose at } 30-\text{ min}) \times 0.25\\ &+ (\text{blood glucose at } 30-\text{ min} + \text{blood glucose at } 60-\text{ min})\\ &\times 0.25\\ &+ (\text{blood glucose at } 60-\text{ min} + \text{blood glucose at } 120-\text{ min})\\ &\times 0.5.\end{aligned}$$

Three days after GTTs, insulin tolerance tests (ITTs) were performed by insulin intraperitoneal injection (1 U/kg body weight) in rats after a 4-h fasting, followed by blood collection at 0 (before injection), 30- and 60-min after insulin injection. Blood glucose was determined as described above.

Serum insulin values were determined by radioimmunoassay, and homeostatic model assessment-insulin resistance (HOMA-IR) scores were calculated from the glucose and insulin values by the following equations: HOMA-IR index = FBG (mmol/L) × Fasting insulin (FINS, units/L) / 22.5 to assess changes in insulin resistance during the experimental period [23].

2.5. Histology examination

At the end of the experiments (three days after ITTs), rats were fasted overnight and euthanized by a sodium pentobarbital overdose (66 mg/kg, intraperitoneal). The hepatic tissues were quickly removed and immerged in 4% paraformaldehyde for 48 h and then dehydrated in gradient ethanol step by step. After embedding in wax, tissues were sectioned at 5 μm thickness using microtome (Leica, Germany) and stained with hematoxylin-eosin (HE) and examined by a light microscope digital camera (Nikon Instruments, Japan).

2.6. Western blot analysis

To clarify the molecular mechanisms of CIHH treatment, the expression of proteins related to glucose metabolism and insulin signaling in the liver were examined. The hepatic tissues were homogenized in a lysis buffer and protein concentration was determined by the Bradford assay (TIANGEN, China). Protein samples (50 μg) were separated by SDS–PAGE, transferred to a PVDF membrane (Millipore Corporation, USA) that was blocked for 1 h with 5% (w/v) non-fat milk in tris-buffered saline, and incubated with antibodies against HIF1α (1:1000, Wanleibio, China), IRS-2 (1:1000, Cell Signaling Technology, USA), HIF2α (1:1000, abcam, UK), IRS-1 (1:1000, Affinity, USA), PEPCK (1:1000, Sangon Biotech, China), and GCK(1:1000, AVIVA SYSTEMS BIOLOGY, USA) overnight at 4 °C. The same membrane was stripped and re-blotted with an anti-β-actin antibody (1:5000, Affinity, USA) for normalization. Blots were developed by the chemiluminescent detection method. The protein blots was quantified by densitometry using NIH image software and normalized to β-actin.

2.7. Data analyses

All data were expressed as means ± S.E.M. Results were assessed using one-way ANOVA followed by SNK test for multiple comparisons. *t*-Test was used for comparison between two groups. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of CIHH on general condition of animals

Compared with CON and CIHH rats, DM and DM + CIHH rats displayed polyphagia, polyuria and weight loss ($P < 0.05$–0.01, Fig. 1). The success rate of achieving the T2DM model in the present study was 75% (at first, sixteen rats were involved in DM and DM + CIHH groups, and twelve T2DM rats were left and used in the experiment).